Figure 1. Nucleotide and amino acid sequence of 60kCRMP from Chalmydia muridium.

-	-			gat Asp		-				_	_	-	_		_	48
1			<u></u>	5					10					15	•	
atc	ttc	aca	ata	act	aαt	ata	aca	aqt	tta	ttt	qct	agc	aaa	ata	tta	96
			Val	Thr				Ser				Ser	Gly			
	•		20		Ψ			25					30			
				gca												144
Glu	Thr	Ser 35	Met	Ala	Glu	Ser	Leu 40	Ser	Thr	Asn	Val	Ile	Ser	Leu	Ala	
												20				
				aaa Lys												192
p	50	<b>1</b> 275	niu	2,5	014	55	****	CCI	1110	GIII	60	пор	nrg	my o	AIG	
ana	222	aat	cat	caa	aat	add	act	tcc	ata	ata	cat	222	nan	att	act	240
				Gln					_	-	_			-		240
65					70					75					80	
gca	gtt	cgt	gat	act	aaa	gct	gta	gag	cct.	aga	cag	gat	tct	tgc	ttt	288
Ala	Val	Arg	Asp	Thr 85	Lys	Ala	Val	Glu		Arg	Gln	Asp	Ser		Phe	
									90					95		•
				aca												. 336
GIY	nys	Mer	100	Thr	vai	гуя	Val	105	Asp	Asp	Arg	Asn	110	GIU	ire	
																204
			-	cct Pro	_											384
•		115				-	120			•		125	•			
gag	att	act	qct	ata	ggg	aaa	aqa	gac	tat	att	gat	σta	atc	att	aca	432
_	Ile		-	Ile		ГÀЗ	_	-	_	_	Asp	-				
	130					135					140					
	_			tgc	-	-			-	_	_	_		-		480
Gln 145	Gln	Leu	Pro	Cys	Glu 150	Ala	Glu	Phe	Val	Ser	Ser	Asp	Pro	Ala	Thr 160	
				gat Asp												528
****	110		nau	165	O <sub>1</sub> y	Ly S	DCu	var	170	פעם	110	vob	nra	175	GLY	
cac	aaia	as a	220	aqt	222	2++	act	at a	traa	at a	222	cot	att		<b>~</b> 22	576
	~-	_	_	Ser				_		-					_	576
			180					185					190			
ggt	tgc	tgc	ttt	aca	gct	gca	acg	gtt	tgt	gct	tgt	cca	gag	atc	cgt	624
GJÀ	Сув	-	Phe	Thr	Ala	Ala		Val	Cys	Ala	Cys		Glu	Ile	Arg	
		195					200					205				•
tcg	gtt	acg	aaa	tgt	ggc	cag	cct	gct	atc	tgt	gtt	aaa	cag	gaa	ggt	672
ser	Val 210	Tnr	гуя	Cys	GТĀ	G1n 215	PLO	Ата	тте	cys	220	гĀS	GIU	GIU	GIÅ	
				L L	<b>L</b> .		<b>.</b>							2	- de -	200
				tgt Cys												720
225				-2-	230	J	- 3			235	-3-	ر			240	

				•													_
					gca Ala											768	3
-		_			gct Ala		_				_	_	_			816	5
			_	_	caa Gln			_	-	_						864	1
					cgt Arg		Arg									912	2
					aaa Lys 310											960	j.
		-			gtt Val					_	-				gtt Val	100	3
					tat Tyr											105	5
					gta Val											110	4
_	_	_	_	_	gga Gly	_	-			_			_	-		. 115	2
					aat Asn 390											120	D
		-			cca Pro							-	_			124	3
_	_		-	_	ggt Gly		_			_	_	-	_			129	5
					gct Ala											134	4
					gga Gly											139	2
					gaa Glu 470											144	0
					cct Pro											148	8

				485					490					495		
att Ile	aca Thr	gga Gly	aac Asn 500.	Thr	gta Val	gtg Val	ttt Phe	gat Asp 505	tcg Ser	tta Leu	cct Pro	aga Arg	tta Leu 510	ggt Gly	tct Ser	1536
aaa Lys	gaa Glu	act Thr 515	gta Val	gag Glu	ttt Phe	tct Ser	gta Val 520	acg Thr	ttg Leu	aaa Lys	gca Ala	gta Val 525	tcc Ser	gct Ala	gga Gly	1584
gat Asp	gct Ala 530	cgt Arg	999 Gly	gaa Glu	gct Ala	att Ile 535	ctt Leu	tct Ser	tcc Ser	gat Asp	aca Thr 540	ttg Leu	aca Thr	gtt Val	cct Pro	1632
gta Val 545	tct Ser	gat Asp	acg Thr	gag Glu	aat Asn 550	aca Thr	cat His	atc Ile	tat Tyr							1662

Figure 2 C. trachomatis equivalent 60kCRMP nucleic acid and amino acid sequence.

				•													
atg Met 1	cga Arc	ata [ Ile	gga Gly	gat Asp 5	cct Pro	atg Met	aac	aaa Lys	ctc Leu 10	ato Ile	aga Arg	cga Arc	gca Ala	gto Val	acg Thr	٠	48
ato Ile	ttc Phe	gcg Ala	gtg Val 20	act Thr	agt Ser	gtg Val	gcg Ala	agt Ser 25	tta Leu	ttt Phe	gct Ala	ago Ser	ggg Gly 30	gto Val	tta Leu		96
gag Glu	acc	Ser 35	atg Met	gca Ala	<b>Ψ</b> gag Glu	tct Ser	ctc Leu 40	tct Ser	aca Thr	aac	gtt Val	att Ile 45	ago Ser	tta Lev	gct Ala		144
gac	acc Thr 50	aaa Lys	gcg Ala	aaa Lys	gac Asp	aac Asn 55	act Thr	tct Ser	cat His	aaa Lys	ago Ser 60	aaa Lys	aaa	gca	aga Arg		192
aaa Lys 65	aac Asn	cac His	agc Ser	aaa Lys	gag Glu 70	act Thr	ccc Pro	gta Val	gac Asp	cgt Arg 75	aaa Lys	gag Glu	gtt Val	gct Ala	ccg Pro 80		240
gtt Val	cat His	gag Glu	tct Ser	aaa Lys 85	gct Ala	aca Thr	gga Gly	cct	aaa Lys 90	cag Gln	gat Asp	tct Ser	tgc Cys	ttt Phe 95	ggc		288
aga Arg	atg Met	tat Tyr	aca Thr 100	gtc Val	aaa Lys	gtt Val	aat Asn	gat Asp 105	gat Asp	cgc Arg	aat Asn	Val	gaa Glu .110	atc Ile	aca Thr	•	336
caa Gln	gct Ala	gtt Val 115	cct Pro	gaa Glu	tat Tyr	gct Ala	acg Thr 120	gta Val	gga Gly	tct Ser	ccc Pro	tat Tyr 125	cct Pro	att Ile	gaa Glu		384
att Ile	act Thr 130	gct Ala	aca Thr	ggt Gly	aaa Lys	agg Arg 135	gat Asp	tgt Cys	gtt Val	gat Asp	gtt Val 140	atc Ile	att Ile	act Thr	cag. Gln		432
caa Gln 145	tta Leu	cca Pro	tgt Cys	gaa Glu	gca Ala 150	gag Glu	ttc Phe	gta Val	cgc Arg	agt Ser 155	gat Asp	cca Pro	gcg Ala	aca Thr	act Thr 160		480
cct Pro	act Thr	gct Ala	gat Asp	ggt Gly 165	aag Lys	cta Leu	gtt Val	tgg Trp	aaa Lys 170	att Ile	gac Asp	ege Arg	tta Leu	gga Gly 175	caa Gln		528
ggc Gly	gaa Glu	aag Lys	agt Ser 180	aaa Lys	att Ile	act Thr	gta Val	tgg Trp 185	gta Val	aaa Lys	cct Pro	ctt Leu	aaa Lys 190	gaa Glu	ggt Gly		5 <b>7</b> 6
tgc Cys	tgc Cys	ttt Phe 195	aca Thr	gct Ala	gca Ala	aca Thr	gta Val 200	tgc Cys	gct Ala	tgt Cys	cca Pro	gag Glu 205	atc Ile	cgt Arg	tcg Ser		624
gtt Val	aca Thr 210	aaa Lys	tgt Cys	gga Gly	caa Gln	cct Pro 215	gct Ala	atc Ile	tgt Cys	gtt Val	aaa Lys 220	caa Gln	gaa Glu	ggc Gly	cca Pro		672
gag Glu 225	aat Asn	gct Ala	tgt Cys	ttg Leu	cgt Arg 230	tgc Cys	cca Pro	gta Val	Val	tac Tyr 235	aaa Lys	att Ile	aat Asn	ata Ile	gtg Val 240		720

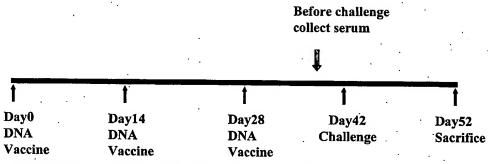
		gga Gly														768
		ggt Gly														816
		gat Asp 275														864
		ctt Leu										Thr				912
		gga Gly														960
cct Pro	tgc Cys	gta Val	caa Gln	gta Val 325	agt Ser	att Ile	gca Ala	gga Gly	gca Ala 330	gat Asp	tgg Trp	tct Ser	tat Tyr	gtt Val 335	tgt Cys	1008
aag Lys	cct Pro	gta Val	gaa Glu 340	tat Tyr	gtg Val	atc Ile	tcc Ser	gtt Val 345	tcc Ser	aat Asn	cct Pro	gga Gly	gat Asp 350	ctt Leu	gtg Val	1056
		gat Asp 355														1104
		gct Ala														1152
gtg Val 385	aaa Lys	gaa Glu	ctg Leu	aat Asn	cct Pro 390	gga Gly	gag Glu	tct Ser	cta Leu	cag Gln 395	tat Tyr	aaa Lys	gtt Val	cta Leu	gta Val 400	1200
		caa Gln														1248
		gac Asp														1296
		gga Gly 435														1344
cct Pro	gtt Val 450	tgt Cys	gta Val	gga Gly	gaa Glu	aat Asn 455	act Thr	gtt Val	tac Tyr	cgt Arg	att Ile 460	tgt Cys	gtc Val	acc Thr	aac Asn	1392
		tct Ser														1440
		ctg Leu														1488

	485							490			495					
			aca Thr 500												1536	
_		_	gag Glu			_	_		_	_	_		_		1584	
			gaa Glu												1632	
	_		gag Glu									•			1659	

Figure 3 Immunization protocol.

## **Protocol**

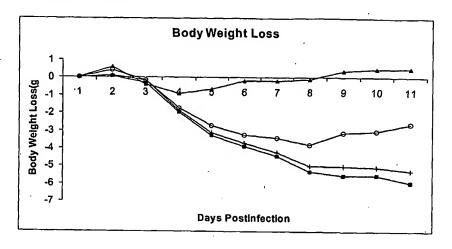
Animal: Female BALB/c mice(4-5weeks old)
: Four to 8 mice per group



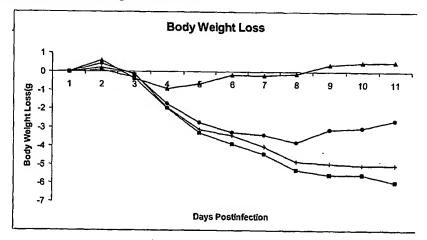
DNA Vaccine: Intranasal 100ug&intramuscular 200ug of plasmid DNA(2ug/ul)

Figure 4 Body Weight loss after immunization.

Panel A 60kCRMP full-length



Panel B 60kCRMP signal sequence deleted

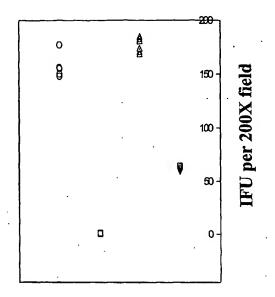


## **▲** - EB

- o-pCACT CRMP60k
- - pCACT CRMPdelta
- + pCAMycHis
- Naive

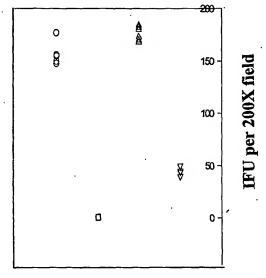
Figure 5 Clearance of Chlamydia from the Lungs of Immunized Mice.

Panel A 60kCRMP full-length



(p<0.001)

Panel B 60kCRMP signal sequence deleted



(p<0.001)

o- Naïve,

 $\Box$  – EB,

 $\Delta$  - pCAMycHis,

∇ - pCACT CRMP60k (Panel A)

- pCACT CRMPdelta

Figure 6. Plasmid pET30b(+)60kCRMP+SP cloning Procedure.

